608-Pos Board B394

The Mechano-Activated Potassium Channel from Human Erythrocyte (HEMKCA): Effect of Oxidative Stress and its Implication on the Senescence Process

Jesus G. Romero, Angeles R. Zambrano.
Universidad Central Venezuela, Caracas, Venezuela, Bolivarian Republic of.

Human erythrocyte (hRBC) shows a tightly controlled programmed cell death with a live span of 120 days. But, as these cells lack nucleus and organelles several question arises: Which are the molecular entities behind this biological clock? How is this process controlled? A widely held hypothesis proposes that hRBC membranes at microcirculation level are exposed to mechanical shear, and somehow these periodical changes lead to the senescence process of these cells. Using Patch Clamp we had characterized a mechano-activated K^+ channel (HEMKCA), which shows a sigmoid dependence of *Po* on applied pressure, a mean conductance of 17pS, and is Ca^{2+} modulated (*140mM*KCl*,10mM*NaCl*,1mM*$CaCl_2$*,pH*7.0)(1,2), We had proposed a new hypothesis for the process of senescence, with this channel as the molecular entity behind the biological clock. At the microcirculation level, by means of the O_2/CO_2 interplay, these membranes are exposed to an oxidative stress. Here, we present the effects of the oxidazing agent *tert*-Butyl hydroperoxide on the HEMKCA activity. The *Po* of the channel is dramatically reduced by approximately one order of magnitude, this effect being dependent on the concentration of the oxidizing agent (0μM to 25μM). However the burst mode activity, normally at 17.43 ± 17.15 events/burst, gets diminished only by approximately 25%, similarly, the open dwell time presents only a fall of 40% under the same conditions. Although there is an effect on the intraburst variables, the dramatic effect on *Po* seems more likely to be related to the opening mechanism. Here we present a partial kinetic model for this channel, and the oxidative stress is presented as a downregulation mechanism for the senescence process of the Human Red Blood Cell.

(1)(2005) Biophys. J.88(1):593.
(2)(2008) Biophys. J.91(1):1101.

609-Pos Board B395

Exploring the Co-Mutlimerization of bCNG Channels

Hannah R. Malcolm[1], Donald E. Elmore[2], Joshua A. Maurer[1].
[1]Washington University in St. Louis, St. Louis, MO, USA,
[2]Wellesley College, Wellesley, MA, USA.

The bacterial cyclic nucleotide gated (bCNG) channel family is a subfamily of the mechanosensitive channel of small conductance (MscS) superfamily. Members of the bCNG channel family have been shown to gate in response to cyclic adenosine monophosphate alone, but are incapable of rescuing *E. coli* in osmotic downshock assays. In the bCNG channel family, some bacterial genomes encode for multiple bCNG homologues while others encode for a single homologue. The presence of multiple bCNG homologues in a single bacterial genome has lead us to hypothesize that these channels might exist as heteromultimers, which would represent the first observation of heteromultimeric channels in prokaryotes. In a pull-down assay, bCNG homologues from a single bacterial genome, form heteromultimers, when heterologously expressed in *E. coli*. Additionally, bCNG channels from different bacteria can form heteromultimers when co-expressed with bCNG channels containing the same number of transmembrane domains. To determine if bCNG heteromultimers are formed *in vivo*, RT-PCR was used to verify the co-translation of bCNG channels.

610-Pos Board B396

Structural and Mechanistic Insights into Gating of K2P Channels

Markus Rapedius[1], Paula L. Piechotta[2], Philip J. Stansfeld[3], Murali K. Bollepalli[1], Gunter Ehrlich[2], Isabelle Andres-Enguix[4], Hariolf Fritzenschaft[1], Niels Decher[5], Mark S.P. Sansom[3], Stephen J. Tucker[4], Thomas Baukrowitz[1].
[1]Institute of Physiology, University of Kiel, Germany, [2]Institute of Physiology II, University of Jena, Germany, [3]Structural and Computational Bioinformatics Unit, Department of Biochemistry, University of Oxford, United Kingdom, [4]Department of Physics, Clarendon Laboratory, University of Oxford, United Kingdom, [5]Institute of Physiology and Pathophysiology, University of Marburg, Germany.

Two-pore domain (K2P) potassium channels are important regulators of cellular electrical excitability and are capable of integrating a wide range of signalling pathways. However, the structure of these channels and their gating mechanism, in particular the role of the helix-bundle-crossing gate, are not well understood. We recently reported that quaternary ammonium (QA) ions bind with high-affinity to several different K2P channels and can be used as a tool to probe their inner pore structure and to explore their gating mechanisms itself. Our results identify a QA ion-binding site deep within the pore of TREK-1 and suggest that the pore structure of this channel most closely resembles the open-state structure of KvAP. We also used the slow binding kinetics of large QA ions (e.g. tetrahexylammonium) to investigate the pH- and pressure-gating mechanisms of TREK-1. Interestingly, these studies suggested that the helix-bundle-crossing gate remains open even when the channel is closed. We also found that mutations close to the selectivity filter and the nature of the permeant ion (K^+, Rb^+ or Tl^+) both had a profound influence upon intracellular pH-gating suggesting that the primary gating mechanism in K2P channels resides close to or within the selectivity filter. Here, we further investigate the status of the helix-bundle gate and structural changes in the pore for several gating principles in K2P channels. To this end we introduced cysteins in TM2 and TM4 of TREK-1 and probed the state dependence of their chemical modification. Furthermore we will report about the intimate coupling of gating and permeation in K2P channels by exploring the effect of different ions and voltage protocols.

611-Pos Board B397

The Hollow Domain of the Mechanosensitive Channel MscS is a Sensor of Cytoplasmic Crowding

Ian Rowe, Kishore Kamaraju, Miriam Boer, Vladislav Belyy, Andriy Anishkin, Sergei Sukharev.
University of Maryland, College Park, MD, USA.

Mechanosensitive channel MscS is a ubiquitous osmolyte release valve found in walled cells, from bacteria to higher plants. In excised patches, applied tension of 5-8 mN/m activates *E. coli* MscS, followed by adaptive closure and complete inactivation, both of which are strongly promoted by crowding agents in the cytoplasm and by depolarizing voltages. Previous genetic screens[1] and models derived from the crystal structure suggest allosteric coupling between the cytoplasmic 'cage' domain and the gate through association of the beta domain and the cytoplasmic end of the pore-lining TM3 helix. This association imposes kinks on TM3 that lead to closing or inactivation. Our analysis shows that mutations disrupting the TM3-beta association dramatically decrease closing rates, impede inactivation and make the channel insensitive to crowding agents and voltage. Conversely, mutations predicted to strengthen the TM3-beta interactions make the channel hard to open and inactivate silently under ramp stimulation. Models with straightened TM3s and separated beta domains representing the resting and open states are supported by an engineered salt bridge, which was not observed in the crystal structure and strongly destabilized the open state. Simulations suggest that in the resting state, with beta domains separated from TM3, the cage is prolate in shape, protruding more into the cytoplasm, whereas conversion to an oblate shape and approaching the membrane favors TM3b-beta association. We conclude that the shape changes make the cage an intracellular crowding sensor which functions through labile kink-stabilizing TM3-beta interactions. The cage is the first example of a hollow channel domain that can provide feedback on the degree of cytoplasm condensation (crowding) that disengages the gate and prevents efflux of osmolytes.

1. Koprowski P, Grajkowski W, Isacoff EY and Kubalski A. 2011. *J Biol Chem.* 286:877-88.

612-Pos Board B398

A Model of the Open Pore MscL Based on Experimental Data and Restrained Coarse Grained Simulations

Evelyne Deplazes[1], Dylan Jayatilaka[1], Martti Louhivuori[2], Siewert-Jan Marrink[2], Ben Corry[1].
[1]University of Western Australia, Crawely, Australia, [2]University of Groningen, Groningen, Netherlands.

Mechanosensitive channels are ubiquitous membrane proteins where gating is induced by tension in the lipid bilayer. A high resolution structure of the closed pore of the mechanosensitive channel of large conductance (MscL) is known and the protein has been characterized in different functional states using EPR [1] and FRET [2] spectroscopy. However, details of the open-channel structure and the gating mechanism remain unknown.

We present an open pore model obtained from restrained simulations in which inter-subunit distances [1,2] and solvent accessibility data [2] were incorporated into a coarse grained model of MscL. 2 μs simulations combining restraints and tension were carried out. The open-channel structures show a pore diameter between 26Å and 32Å and TM1 and TM2 tilts of ~60° and ~46°. These measurements are in good agreement with data from previously reported open pore models. In contrast, there is little evidence of rotation of the TM helices. In all simulations the N-terminal lies along the membrane surface making it unlikely to serve as a second gate. The C-terminal does not dissociate during gating but shows indication of upward motion that may stabilize the open state. Results further suggest the open pore the opening is associated with an outward motion of the periplasmic loop in combination with the formation of a kink in the periplasmic end of TM1 and tension-induced thinning of the membrane is necessary for these structural changes to occur.